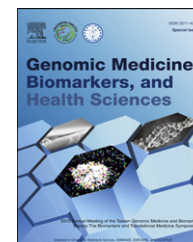


Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-gmbhs.com

SHORT COMMUNICATION

Internal transcribed spacer sequence analysis of *Angelica* from different habitats

Hsiao-Chun Hao^a, Jia-Yuan Chang^b, Fu-Yen Chung^{b,*}^a Department of Laboratory Medicine, Division of Laboratory Medicine, Fooyin University Hospital, Pingtung, Taiwan^b Division of Medical Research, Fooyin University Hospital, Pingtung, Taiwan

Received 13 March 2012; accepted 30 March 2012

Available online 11 May 2012

KEYWORDS*Angelica*;
DNA sequence;
internal transcribed
spacer (ITS)

Abstract The internal transcribed spacer (ITS), located between the 18S and 26S nuclear ribosomal DNA (rDNA) sequences, has a high degree of variation. Analysis of ITS sequences is commonly used to identify the authenticity of Chinese herbal medicines. The aim of this study is to analyze ITS sequences of *Angelica* from different habitats to find out whether there are differences in sequence. *Angelicas* from three habitats were used in this study, including Taiwan, Sichuan Province (China), and Gansu Province (China). DNA was extracted from *Angelicas*, and ITS sequences were analyzed using polymerase chain reaction (PCR) and direct sequencing. The results showed that the similarity of ITS-1 and ITS-2 rDNA sequences in *Angelicas* produced in Gansu and Sichuan Provinces is up to 100%, and that produced in Taiwan and Sichuan Province is 88% and 87%, respectively. Therefore, we could use PCR and ITS sequencing analysis to identify whether these *Angelicas* were the same strain, in order to determine their worthiness and authenticity in terms of traditional Chinese medicine.

Copyright © 2012, Taiwan Genomic Medicine and Biomarker Society. Published by Elsevier Taiwan LLC. All rights reserved.

Introduction

The authenticity and quality level of a Chinese herbal medicine often affect its efficacy. *Angelica* is a popular

Chinese herbal medicine which is also added to dishes as a tonic. Among various strains of *Angelicas*, those produced in Sichuan Province of China are the most famous ones because of their high quality. The internal transcribed spacer (ITS) region, located between 18S and 26S nuclear ribosomal DNA (rDNA) sequences, contains a highly conserved and variable region and, therefore, is frequently used to identify the authenticity of Chinese herbal medicines.^{1–3} In this study, we analyzed the ITS sequences of *Angelicas* from various habitats to identify whether there are any differences in their sequences.

* Corresponding author. Division of Medical Research, Fooyin University Hospital, No. 5, Jhongshan Road, Donggang Township, Pingtung 928, Taiwan.

E-mail address: fuyenchung@gmail.com (F.-Y. Chung).

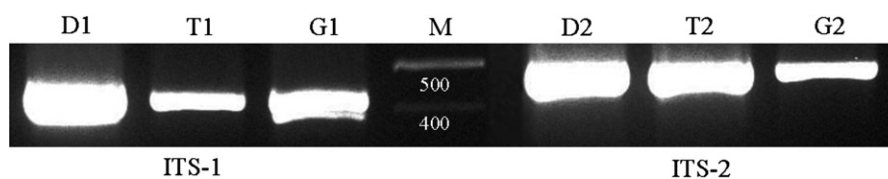


Figure 1 PCR analysis of ITS-1 and ITS-2 sequences. Length of ITS-1 and ITS-2 fragments is 400 and 450 bps, respectively. D1 and D2 represent *Angelica* from Sichuan province, China; T1 and T2 represent *Angelica* from Taiwan; and G1 and G2 represent *Angelica* from Gansu province, China. ITS = internal transcribed spacer; PCR = polymerase chain reaction.

Materials and methods

Two samples of *Angelica sinensis* (Gansu and Sichuan Provinces, China) and one sample of *Angelica acutiloba* (Taiwan) were used in this study. *A. sinensis* was purchased from a Chinese herbal medicine store, and *A. acutiloba* was purchased from Yuf Biotechnology Company (Pingtung, Taiwan). DNA was extracted from *Angelicas* using a Pure-gene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Sequences of polymerase chain reaction (PCR) primers were designed by Primer3 (v. 0.4.0) (<http://frodo.wi.mit.edu/>). PCR was carried out at a final concentration of $1 \times$ PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; and 2 mM $MgCl_2$), 50 mM deoxyribonucleotide triphosphate (dNTP), 0.1 mM of the oligonucleotide primers (ITS-1 forward: 5'-CGCGAGAAGTCCACTAAACC-3', reverse: 5'-TTGCGTTCAAAGACTCGATG-3'; ITS-2 forward: 5'-GATATCTCGGCTCTCGCATC-3', reverse: 5'-CTCGCCGTTACTAGGGGAAT-3'), and 2.5 U Tag DNA polymerase (Promega Corp, Madison, WI, USA) in a total volume of 50 μ L. PCR amplification was carried out in a BIO-RAD PT-200 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA), under the following conditions: initial denaturation at 95 °C for 5 minutes; 35 cycles at 94 °C for 30 seconds, at 60 °C for 30 seconds, at 72 °C for 1 minute, and then at 72 °C for 5 minutes for the final extension. The product of PCR amplification was purified using DNA clean/extraction kit (GeneMark Technology, Tainan, Taiwan), according to the manufacturer's instructions. Analysis of ITS-1 and ITS-2 sequences was commissioned to sequencing services provided by Tri-I Biotech Inc. (Tri-I Biotech Inc, Taipei, Taiwan).

Results

The results from PCR analysis showed the length of the ITS-1 and ITS-2 fragments to be 400 and 450 bps, respectively (Fig. 1). The sequencing analysis showed that the ITS-1 and ITS-2 rDNA sequences in *Angelica* strains from Sichuan and Gansu Provinces are 100% identical, indicating that *Angelicas* from these two provinces are from the same strain. ITS-1 and ITS-2 rDNA sequences between *Angelicas*

from Taiwan and Sichuan showed similarity of 88% and 87%, respectively, indicating that the two are not from the same strain.

Discussion and conclusion

The gene sequence of plant nuclear ribosomal DNA showed a high degree of homology within the same species, with small evolutionary variability. The ITS sequences of rDNA contains variable regions and have been considered as powerful genetic markers for the identification of closely related species.^{4,5} Analyses of ITS sequences are often used to identify different species for appraising the origin of the Chinese herbs that are morphologically similar. In China, *A. sinensis* grows mostly in places such as Gansu, Sichuan, and Yunnan Provinces. In Taiwan, *A. acutiloba* grows mostly in the rural areas of Hualien County, including Yuli, Zhuoxi, Rueisuei, and Kuangfu. Sequence analysis of this study confirmed that ITS-1 and ITS-2 sequences of *A. sinensis* are different from that of *A. acutiloba*. These ITS sequences act as genetic markers for the identification of plant species and, thus, are used to identify one of the favorable evidences for the authentication of Chinese herbal medicine.

References

1. Lin LZ, Harnly JM. Identification of hydroxycinnamoylquinic acids of arnica flowers and burdock roots using a standardized LC-DAD-ESI/MS profiling method. *J Agric Food Chem*. 2008;56:10105–10114.
2. Monod M, Bontems O, Zaugg C, et al. Fast and reliable PCR/sequencing/RFLP assay for identification of fungi in onychomycoses. *J Med Microbiol*. 2006;55:1211–1216.
3. Song Z, Wang X, Liang G. Species identification of some common necrophagous flies in Guangdong province, southern China based on the rDNA internal transcribed spacer 2 (ITS2). *Forensic Sci Int*. 2008;175:17–22.
4. Wu P, Zhou K, Zhang Z, et al. Molecular genetic marker identification of traditional Chinese drug hippocampus. *Yao Xue Xue Bao*. 1998;33:226–233.
5. Yang BB, Guo XG, Hu XS, et al. Species discrimination and phylogenetic inference of 17 Chinese Leishmania isolates based on internal transcribed spacer 1 (ITS1) sequences. *Parasitol Res*. 2010;107:1049–1065.